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EVALUATION OF ANTIMICROBIAL ACTIVITY OF DIVYA DHOOPA CHURNA AND ITS MODIFIED DIVYA DHOOPA STICKS

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ABSTRACT

References about communicable diseases in Ayurvedic classics are elaborated under the heading *Janapadodhwamsa*. In recent years, communicable diseases have been a major threat to humanity. Dhoopana is one of the treatment modalities mentioned for the treatment of Janapadodhwamsa. Dhoopana Karma is basically done as a part of purification but has therapeutic utility. COVID-19 was an example of the same in recent times where disinfection is proven to be effective, inhibiting the growth of microbes present in the atmosphere. Various modification of Dhoopa Churna was attempted by addition of various other drugs which can help in complete burning of Dhoopa Varti. Antimicrobial study was conducted on three microbes. Complete burning of Dhoopa stick was achieved by addition of sawdust. Arka was prepared after many trials. Antimicrobial fumigation by DDC study showed significant result in inhibiting the colonial growth when compared to DDS. Modification using sawdust was successful as there was complete burning of the stick. Fumigation study was found to be most effective on S.aureus, P.aureginosa and C.albicans when done with Dhoopa Churna.

KEYWORDS: Fumigation, Divya Dhoopa Churna, Anti – microbial, Modification.

INTRODUCTION

Ayurveda emphasizes on positive health and prevention of disease, focuses on well being of individuals.[1] In recent years, communicable diseases have been a major threat to humanity. COVID-19 was an example of the same in recent times where disinfection is proven to be effective, inhibiting the growth of microbes present in the atmosphere.

References about communicable diseases in Ayurvedic classics are elaborated under the heading Janapadodhwamsa[2], means a large population being affected or threatened by certain pathogens or infections with a higher speed of spread which may lead to increased fatality. Dhoopana is one of the treatment modalities mentioned for the treatment of Janapadodhwamsa. Dhoopana Karma is basically done as a part of purification but has therapeutic utility. We can find innumerable references of various Dhoopa Yoga having vast therapeutic Utility in classics of Ayurveda.

One such reference available in Bheshaja Samhitha is Divya Dhoopa Churna(DDC).[3] The main indication of *Divya Dhoopa* is *Janapadodhwamsa*. Other indications include Kasa, Swasa, Hikka and Hridroga. Modification of dosage form is the need of the hour as conventional method of Dhoopana is tedious and time consuming. There are many modified Dhoopa sticks/cones available in the market, which are having chemicals for easy ignition and complete burning of sticks. Hence the study aimed at modifying the Dhoopa Churna into a chemical free yet effective Dhoopa Varti. An attempt was made to assess the Antimicrobial activity of DDC and its modified forms against selected microbes.

OBJECTIVES OF THE STUDY

1. To carryout Pharmaceutico Analytical study of *Divya Dhoopa Churna.*
2. Modification of the *Divya Dhoopa Churna* will be attempted with various possible forms like *Dhoopa* stick, *Dhoopa Varti* etc.
3. To carryout In-vitro study to assess the antimicrobial activity of prepared *Divya Dhoopa Churna* and *Divya Dhoopa Varti* on different strains of Bacteria and fungus.

MATERIALS AND METHOD

Source of Data: Review of literature related to the thesis work was done from different texts of Ayurveda and also online for detailed review of latest updates. Various trials were done for the modification of the DDC.

Drug Source: The raw materials required for preparation of DDC was procured from Amrit Kesari Depot; authentification was done from the Department of Dravya Guna, Sri Sri College of Ayurvedic Science and Research, Bengaluru.

Pharmaceutical source: The preparations were carried out at teaching pharmacy of the department of Rasa Shastra and Bhaishajya Kalpana, Sri Sri College of Ayurvedic Science and Research, Bengaluru.

PHARMACEUTICAL STUDY

PREPARATION OF DDC

Ingredients of DDC includes

Musta	4g	Baala	4g
Tila	4g	Tila Taila	4g
Shati	3g	Raala	3g
Bola	2g	Sita	2g
Nisha	2g	Jatamamsi	2g
Shweta Chandana	5g	Tamalapatra	10g
Guggulu	75g		

- Yavakuta Churna of all ingredients was weighed individually in above said quantity. These Dravya were mixed to form homogenous mixture. To this, Tila Taila was added and mixed thoroughly. Final product was dried in shade. DDC was stored in an air tight container.

PREPARATION OF DIVYA DHOOPA STICKS (DDS)

Various modifications were attempted to convert the DDC into DDS by addition of natural ingredients also ensuring complete burning of the Varti/Stick. Complete burning was achieved in sample prepared by addition of Sawdust in equal quantity to that of Churna.

Table 1: Table showing details of all DDS samples.

Sl.no.	Main Dravya	Additional Dravya Used	Observation and Results
1	Guggulu Paaka + Other Churna + Tila Taila	-	Burnt for less than a minute
2	Guggulu Paaka + Other Churna + Tila Taila	Tila Taila	Did not burn
3	Guggulu Paaka + Other Churna + Tila Taila	Ghrita	Did not burn
4	Guggulu Paaka + Other Churna + Tila Taila	Gomaya Churna	Burnt for 3 minutes
5	Guggulu Paaka + Other Churna + Tila Taila	Karpoora	Burnt for less than a minute
6	Fine powder of Guggulu + + Other Churna + Tila Taila (4.9g)	Karpoora 1 g	Did not burn
7	Fine powder of Guggulu + + Other Churna + Tila Taila(3.5g)	Styrax benzoin (Indian Sambrani/Loban) powder (1.5g)	Burnt for 2 minutes
8	Fine powder of all Dravya Paste made of fine powder of dravya and Tila taila tied in a cloth Dry powder tied in cloth dipped in oil.		Burnt for 20 minutes. Burnt for 19 minutes Both had mild odour of dravya, Burnt cloth smell was more significant
9	Fine powder of Guggulu + Other Churna + Tila Taila(5g)	Charcoal (5g)	Cone – 90% Burnt Varti (Thick and Thin) – Completely burnt Odour of Dravya was significantly less.
10	Fine powder of Guggulu + Other Churna + Tila Taila(5g)	Charcoal (2.5g) + 1g Karpoora	Did not burn
11	Fine powder of Guggulu + Other Churna + Tila Taila(5g)	Charcoal (1g)	Did not burn
12	Fine powder of Guggulu + Other Churna + Tila Taila(3g)	Charcoal (1.5g)	Smaller Varti – Burnt Bigger Varti – Did not burn
13	Fine powder of Guggulu + Other Churna + Tila Taila(3g)	Saw dust (3g)	90% burnt, Odour of Dravyas could be appriciated.

ANALYTICAL STUDY

- Physico chemical analysis[4] of the samples was conducted at Central Research Laboratory of Sri Sri College of Ayurvedic Science and Research, Bengaluru.
- Anti Microbial Fumigation Study of DDC and DDS were conducted on the microbial species Pseudomonas aeruginosa, Staphylococcus aures, Candida albicans at Sri Dharmasthala Manjunatheshwara Centre for Research in Ayurveda and Allied Sciences (AYUSH Centre for Excellence and Recognized SIROS by DSIR) Kuthpady, Udupi.

PROTOCOL FOR EFFICASY STUDY

Preparation of Nutrient agar media to test on Pseudomonas aeruginosa (MTCC 8077)

Beef extract (1 g), yeast extract (2 g), peptone (5 g) and Sodium Chloride (5 g) were dissolved in 990 ml of distilled water. The pH was adjusted to 7.2 and the volume was made up to 1000 ml. Finally 15 g agar was added to the media and autoclaved at 121°C for 20 minutes.

Preparation of Nutrient agar media to test on Staphylococcus aureus (MTCC 3160)

Beef extract (1 g), yeast extract (2 g), peptone (5 g) and Sodium Chloride (5 g) were dissolved in 990 ml of distilled water. The pH was adjusted to 7.2 and the volume was made up to 1000 ml. Finally 15g agar was added to the media and autoclaved at 121°C for 20 minutes.

Preparation of yeast extract dextrose agar media to test on Candida albicans (MTCC 183)

Yeast extract (3 g), peptone (10 g) and dextrose (20 g) were dissolved in 990 ml of distilled water. The pH was adjusted to 7.4 and the volume was made up to 1000 ml. Finally 15 g agar was added to the media and autoclaved at 121°C for 20 minutes.

Preparation of the inoculum

Microbes were procured from Microbial Type Culture Collection and Gene Bank (MTCC), IMTECH, Chandigarh. Loopful of 24 h old culture from the slants was transferred to sterile saline and mixed well to prepare a homogenous inoculum.

Preparation of Buffered Sodium Chloride Peptone Solution (BSCPS) pH 7.0

Dissolve potassium Di Hydrogen Phosphate (3.56 g), Di Sodium Hydrogen Phosphate (7.23 g), Sodium Chloride (4.3 g), Peptone (1.0 g) were taken and dissolved in 990 ml distilled water. The pH was adjusted to 7.0 and make up the volume to 1000 ml. Then above buffer solution was autoclaved at 121°C for 20 minutes.

Procedure

1. 3 Autoclaved petridishes were taken and labelled as half an hour, one hour and without treatment under sterile condition.
2. Media prepared was heated and poured into the petridish.
3. Inoculum was poured to the petridish containing the media and given a homogenous mixture.
4. Petridish sample without treatment was placed inside the Incubator and observed for bacterial growth for 24 hours.
5. Petridish samples with treatment was placed into Fumigation chamber measuring [11"*11"*11"] and Dhoopa Yantra containing the Divya Dhoopa Stick weighing 41.14g was ignited and placed in Dhoopana Yantra and kept in between the chamber.
6. Time was noted and after 30 minutes one of the petridish was placed inside the Incubator and microbial growth was observed for 24 hours.
7. After 1 hour of fumigation another petridish was taken out of the chamber and placed in Incubator and microbial growth was observed for 24 hours.
8. Same procedure was carried out with Divya Dhoopa Churna where Angara was lit and over it 15g of DDC was sprinkled.

RESULTS

ORGANOLEPTIC RESULTS

Table 2: Table showing Organolyptic results.

Sl.no	Sample	Colour	Consistency	Odour
1	DDC	Greenish	Curna	Sugandha
2	DDC+Taila (Guggulu Paka)	Brownish	Varti/Cone	Sugandha
3	DDC+Ghrita (Guggulu Paka)	Brownish	Varti/Cone	Sugandha
4	DDC+Gomaya (Guggulu Paka)	Brownish	Varti/Cone	Sugandha
5	DDC+Karpoora (Guggulu Paka)	Brownish	Varti/Cone	Ishat Karpoora Gandha
6	DDC+Styrax benzoin (Indian Sambrani/Loban) (Guggulu Choorna)	Brownish	Varti/Cone	Characteristic smell of Styrax benzoin (Indian Sambrani/Loban)
7	Cloth method with taila - 1	Brown	Dhoopa choorna smeared cloth	Burnt cloth odour
8	Cloth method with taila - 2	Brown	Dhoopa choorna smeared cloth	Burnt cloth odour
9	DDC(5g) + charcoal (5g)	Black	Varti	Charcoal odour

10	DDC(5g) + charcoal (2.5g+1g Karpoora)	Black	Varti	Charcoal odour
11	DDC(5g) + charcoal (1g)	Black	Varti	Charcoal odour
12	DDC(3g) + charcoal (1.5g)	Black	Varti	Charcoal odour
13	DDC(3g) +Sawdust (3g)	Light brown	Varti	Characteristic odour of DDC

PHYSICO CHEMICAL ANALYSIS RESULTS

The results of physico chemical study conducted as per API standards are as follows:

Table 3: Table Showing Results of Physico- Chemical Analysis.

Parameters tested	Ddc	Dds
Loss On Drying	10.2%	10.4%
Total Ash	8.5%	4%
Water Soluble Ash	1.5%	3.5%
Acid Insoluble Ash	2.5%	1.5%
Ph	6.19	6.58
Water Soluble Extractive	10.8%	5.6%
Alcohol Soluble Extractive	5.2%	2.8%

IN VITRO STUDY RESULT

Analysis of Divya Dhoopa Stick/Varti and Churna Against Pseudomonas Aeruginosa.

Table 4: Table showing microbial analysis of Divya Dhoopa Stick/Varti against Pseudomonas aeruginosa.

SL NO	Dilutions	Incubation Time	No of Colonies (NOC)		CFU/ml
			BATCH 1	BATCH 2	
1	1/1000(10^3)	Without treatment	>300	>300	TNTC*
2	1/1000(10^3)	30 minutes	>300	>300	TNTC*
3	1/1000(10^3)	1 hour	0	0	0

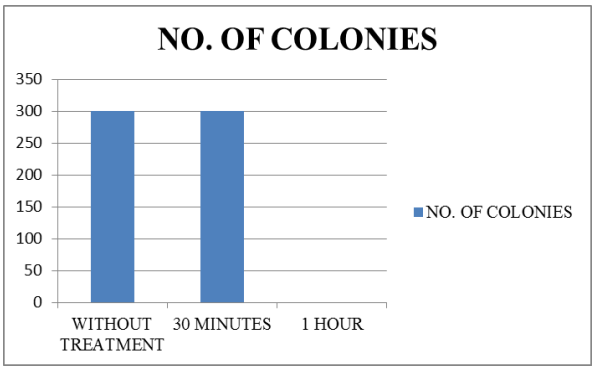

Fig 1: Graph indicating the number of colonies of Divya Dhoopa Stick/Varti against Pseudomonas aeruginosa.

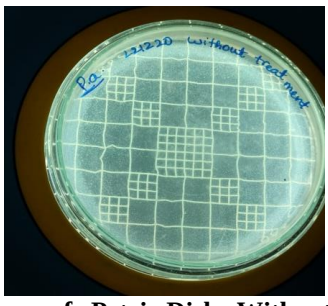

Fig. 2: Image of Petri Dish Without Treatment Against P. Aeruginosa.

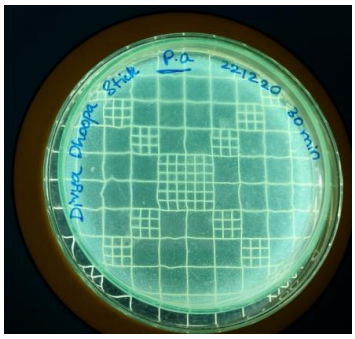

Fig 3: Image after 30 minutes of fumigation with DDS against P.aeruginosa.

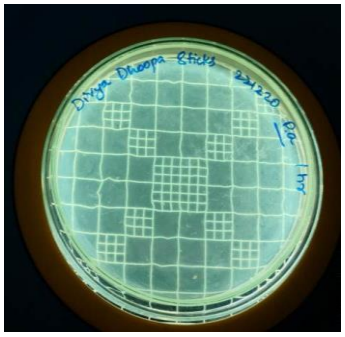

Fig. 4: Image after 1 hour of fumigation with DDS against P.aeruginosa.

Table 5: Table showing microbial analysis of Divya Dhoopa Choorna against *Pseudomonas aeruginosa.*

SL NO	Dilutions	Incubation Time	No of Colonies (NOC)		CFU/ml
			BATCH 1	BATCH 2	
1	1/1000(10^3)	Without treatment	>300	>300	TNTC*
2	1/1000(10^3)	30 minutes	0	0	0
3	1/1000(10^3)	1 hour	0	0	0

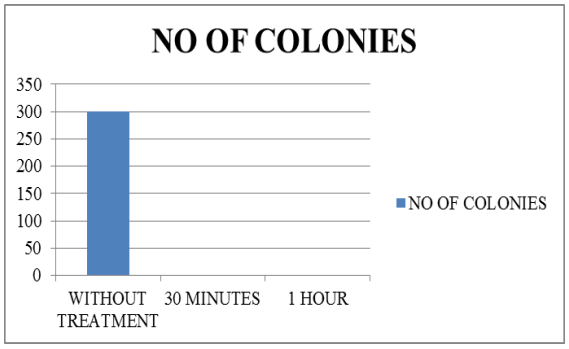

Fig. 5: Graph indicating the number of colonies of Divya Dhoopa Choorna against Pseudomonas aeruginosa.

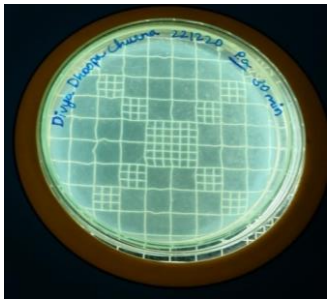

Fig. 6: Image after 30 minutes of fumigation with DDC against P.aeruginosa.

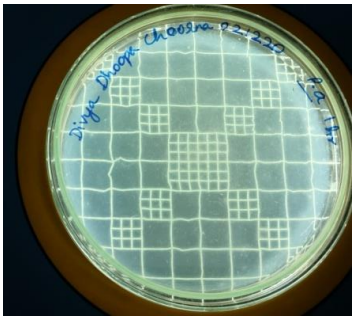

Fig. 7: Image after 1 hour of fumigation with DDC against P.aeruginosa.

ANALYSIS OF DIVYA DHOOPA STICK/VARTI AND CHURNA AGAINST STAPHYLOCOCCUS AUREUS

Table 6: Table showing microbial analysis of Divya Dhoopa Stick/Varti against *Staphylococcus aureus.*

Sl no	Dilutions	Incubation Time	No of Colonies (noc)		CFU/ml
			BATCH 1	BATCH 2	
1	1/1000(10^3)	Without treatment	>300	>300	TNTC*
2	1/1000(10^3)	30 minutes	>300	>300	TNTC*
3	1/1000(10^3)	1 hour	0	0	0

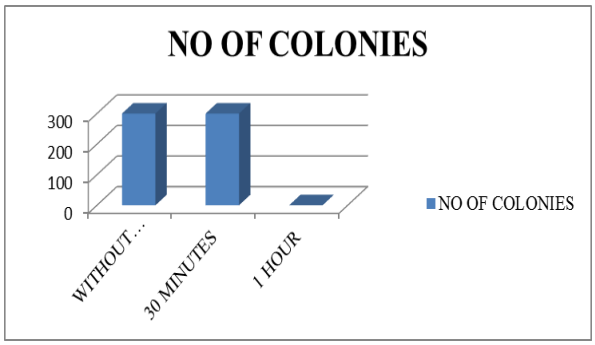

Fig 8: Graph indicating the number of colonies of Divya Dhoopa Stick/Varti against *Staphylococcus aureus.*

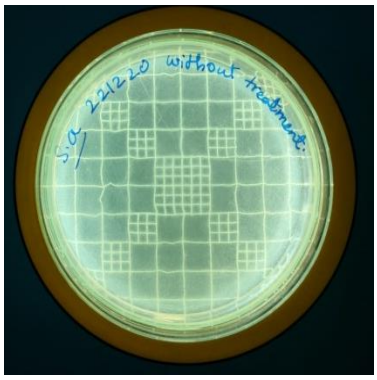

Fig 9: Image of Petri dish without treatment against *Staphylococcus aureus.*

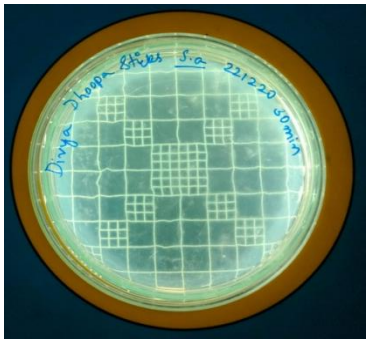

Fig 10: Image after 30 minutes of fumigation with DDS against *Staphylococcus aureus.*

Table 7: Table showing microbial analysis of Divya Dhoopa Churna against *Staphylococcus aureus.*

SL No	Dilutions	Incubation Time	No of Colonies (NOC)		Cfu/ml
			BATCH 1	BATCH 2	
1	1/1000(10^3)	Without treatment	>300	>300	TNTC*
2	1/1000(10^3)	30 minutes	0	0	0
3	1/1000(10^3)	1 hour	0	0	0

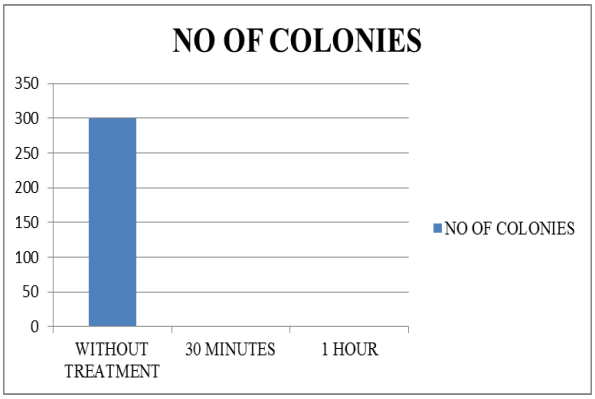

Fig. 11: Graph indicating the number of colonies of Divya Dhoopa Churna against *Staphylococcus aureus.*

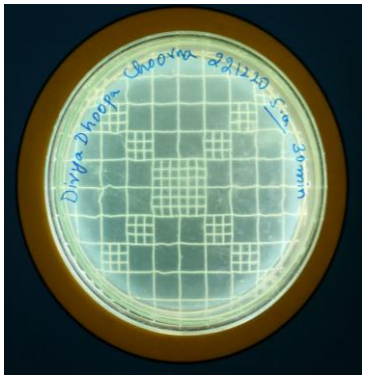

Fig. 13: Image after 30 minutes of fumigation with DDC against *Staphylococcus aureus.*

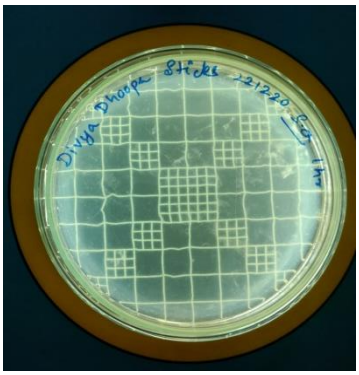

Fig. 12: Image after 1 hour of fumigation with DDC against *Staphylococcus aureus.*

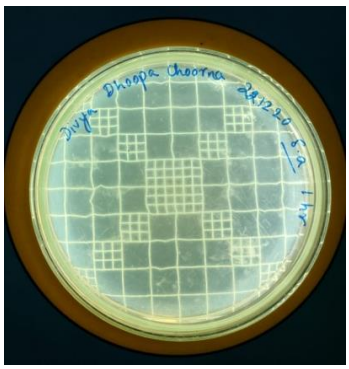

Fig. 14: Image after 1 hour of fumigation with DDC against *Staphylococcus aureus.*

ANALYSIS OF DIVYA DHOOPA STICK/VARTI AND CHURNA AGAINST CANDIDA ALBICANS

Table 8: Table showing microbial analysis of Divya Dhoopa Stick/Varti against *Candida albicans.*

Sl no	Dilutions	Incubation Time	No of Colonies (NOC)		Cfu/ml
			BATCH 1	BATCH 2	
1	1/1000(10^3)	Without treatment	>300	>300	TNTC*
2	1/1000(10^3)	30 minutes	>300	>300	TNTC*
3	1/1000(10^3)	1 hour	0	0	0

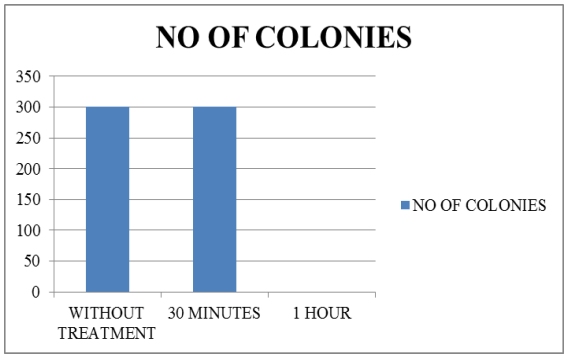

Fig. 15: Graph indicating the number of colonies of Divya Dhoopa Varti/Stick against *Candida albicans.*

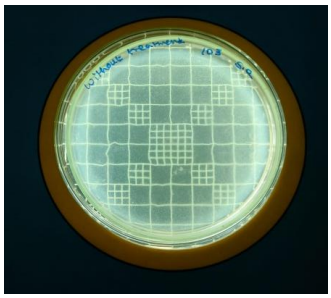

Fig 16: Image of Petri dish without treatment against *Candida albicans.*

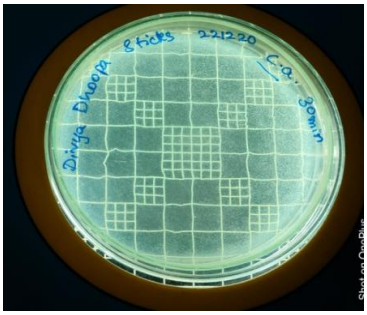

Fig 17: Image after 30 minutes of fumigation with DDS against *Candida albicans.*

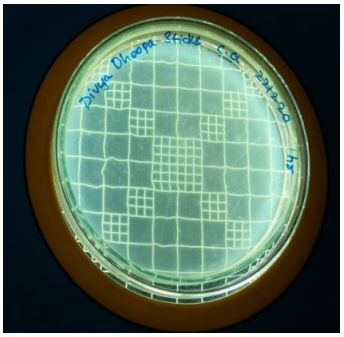

Fig 18: Image after 1 hour of fumigation with DDS against *Candida albicans.*

Table 9: Table showing microbial analysis of Divya Dhoopa Churna against *Candida albicans.*

SL NO	Dilutions	Incubation time	No of Colonies (NOC)		CFU/ml
			BATCH 1	BATCH 2	
1	1/1000(10^3)	Without treatment	>300	>300	TNTC*
2	1/1000(10^3)	30 minutes	>300	>300	TNTC*
3	1/1000(10^3)	1 hour	0	0	0

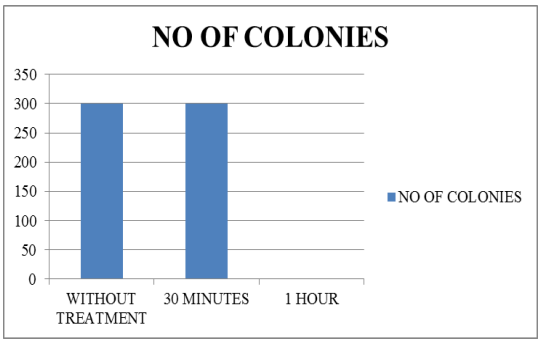

Fig. 19: Graph indicating the number of colonies of Divya Dhoopa Choorna against *Candida albicans.*

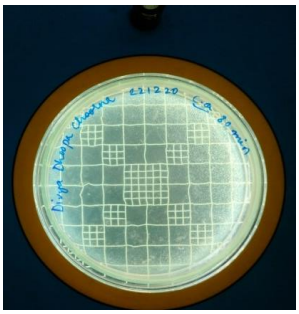

Fig. 20: Image after 30 minutes of fumigation with DDC against *Candida albicans.*

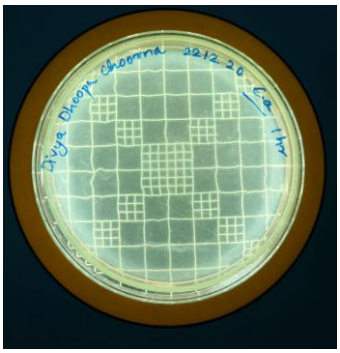

Fig. 21: Image after 1 hour of fumigation with DDC against *Candida albicans.*

DISCUSSION

Concept of Janapadodhwamsa has been extensively studied and many effective treatment protocols have been implemented for the same. Among these Dhoopa Kalpana has a vivid role to play. It has both preventive as well as curative role. In today's scenario we can find various kinds of infectious diseases affecting the mankind.

Divya Dhoopa Churna is a unique formulation explained in Bheshaja Samhita[3] indicated for Janapadodhwamsa.

The drugs included in the formulation are easily available and many of them have been proven of having anti microbial activity.

Most of the ingredients of the Divya Dhooopa Churna are proven to have effective antimicrobial activity. When all these Dravyas having same qualities are used together, they acts synergistically thus increasing the potency of DDC.

Use of Charcoal powder helped in complete burning of the Varti/Cone. But major disadvantage of using this method was that very less or no odour of the drugs of Divya Dhoopa. Also Varti sticks burnt only when equal quantity of Charcoal was added. This might reduce the efficacy of the drugs. When quantity of charcoal was reduced there was incomplete burning of Varti. According to studies high level of Carbon monoxide is produced, especially during the burning of charcoal. Thus usage of Charcoal mixed Dhoopa might be harmful when used for a longer duration.[4] The sample which was prepared using saw dust the odour of Divya Dhoopa was highly appreciated and complete burning was observed.

In vitro Analysis of DDC and DDS

In vitro analysis of DDC and DDS was carried out on the microbes Staphylococcus aureus, Pseudomonas aeruginosa and Candida albicans. Selection of these organisms was to prove the efficacy of the drug against gram positive bacteria, gram negative bacteria and fungus. Selection of these three species was because they are the most commonly found pathogens that can cause diseases in human respiratory system and also because of its easy availability. *S. aureus* and *P. aeruginosa* are responsible for most of the respiratory tract infections and hospital induced disorders. Thus efficacy of DDC on these microbes where tested in in-vitro study.

During anti microbial fumigation with DDS, there was a mild fume observed in the beginning. The fumigation chamber was full of smoke after 20 minutes. This might be the probable reason of growth of microbes after 30 minutes of fumigation in case of *C. albicans S. aureus* and *P. aeruginosa.* Due to long time exposure of the fumes which got intensified may have inhibited the growth of microbes. There was complete growth inhibition after 1 hour of fumigation in all the three samples showing its susceptibility.

In case of DDC dense fumes were observed after inside the fumigation chamber after 5 minutes of starting the procedure. This may be because of larger surface area of Angara over which the Churna was sprinkled. Hence the results showed no growth of microbes after 30 minutes and one hour of fumigation in case of *S. aureus* and *P. aeruginosa* with DDC. There was no growth inhibition in *C. albicans* after 30 minutes of fumigation. However there was no fungal growth after 1 hour.

The addition of saw dust might have reduced the efficacy of the combination thus the anti microbial study shows its efficacy after 1 hour of fumigation. Previous studies have found the anti bacterial as well as anti fungal activity of the ingredients of DDC.

CONCLUSION

Present study on Divya Dhoopa Churna from Bheshaja Samhita was carried out with two objectives. The first being, modification of Divya Dhoopa Churna into other dosage forms which are user friendly and without addition of any chemicals. Second objective of the study was to evaluate its efficacy against few disease causing microbes. Attempt was made to modify the Churna into Dhoopa Varti. Various trials were done to ensure complete burning of Dhoopa Varti without addition of any chemicals. This complete burning was achieved by addition of sawdust powder. Anti microbial fumigation study was performed in a glass chamber using three selected species with Divya Dhoopa Churna and Divya Dhoopa Varti/Stick. The result obtained showed growth inhibition of microbial colonies. Among these two samples Churna exhibited exemplary result when compared to Dhoopa Varti.

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